Exploration of structure-activity relationships of a kinase-targeting chemotype that suppresses *Trypanosoma brucei*.

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Exploration of structure-activity relationships of a kinase-targeting chemotype that suppresses *Trypanosoma brucei*.

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A thesis submitted to

The Faculty of
the College of Science of
Northeastern University
in partial fulfillment of the requirements
for the degree of Master of Science

April 24, 2015

Thesis directed by
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Professor of Chemistry and Chemical Biology
Acknowledgements

My time at Northeastern University has been an incredible experience that has shaped me both academically and personally. I could not be where I am today without the help of a number of people. My advisor Dr. Michael Pollastri is at the top of my list to thank, as he has served as a guide not only through my research but through my professional journey as well. Dr. Pollastri has been very patient with me as I decided where my career interests lie. I truly appreciate the advice, support, and funding he has given me throughout the years.

It has been a pleasure to work with all members of the Pollastri lab, past and present. Alumni group members Dr. Gautum Patel, Dr. Emanuele Amata, Dr. Jennifer Woodring, Dr. David Finnegan, and especially my first mentor Dr. Stefan Ochiana welcomed me to lab and showed me the ropes. I would like to thank William Devine for acting as an unofficial mentor, and Naimee Mehta, Dana Klug, and Dr. Seema Bag for being such great friends both in and out of lab. Lisseth Silva has been a huge influence in my life and has turned into one of my best friends, giving me the support I needed. Working with such wonderful people, the undergraduate and master’s students, truly made the lab an enjoyable workplace.

I would also like to thank Dr. Graham Jones and Dr. James Aggen for being members of my thesis committee and for giving me professional guidance and input, and the Department of Chemistry and Chemical Biology at Northeastern for the opportunity. I have been especially fortunate to have the opportunity to be a part of the IGERT program. With Dr. Srinivas Sridhar and Dr. Anne van de ven-Maloney’s assistance, I was able to develop a different skill set by learning about nanomedicine. I greatly appreciate the training and funding I received from the IGERT fellowship.
Abstract

Human African trypanosomiasis is a lethal neglected tropical disease in need of more effective and efficient treatment. In an effort to develop inhibitors of the parasite that causes the disease, *Trypanosoma brucei*, data from a high throughput screen run in collaboration with GlaxoSmithKline was analyzed. Using an algorithm-based scoring system, several analogs were designed and synthesized to optimize the physical chemical properties while maintaining potency. The structure-activity relationship was evaluated from several synthesized analogs and additional analogs have been proposed for further studies.
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List of Abbreviations

ClogD  calculated distribution coefficient at pH = 7.4
ClogP  calculated partition coefficient
CNS   central nervous system
DALY  disability-adjusted life years
DCM   dichloromethane
DEA   diethylamine
DIPEA diisopropylethylamine
DMA   dimethylamine
DMAP  4-dimethylaminopyridine
DME   dimethoxyethane
DMF   dimethylformamide
EDC   1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
$^1$H NMR proton nuclear magnetic resonance
HAT   human African trypanosomiasis
HBD   hydrogen bond donor
HepG2 human liver carcinoma cell line
HOBt  hydroxybenzotriazole
HTS   high throughput screen
IC$_{50}$ half maximal carcinoma cell line
GSK   GlaxoSmithKline
LC-MS liquid chromatography-mass spectrometry
mCPBA meta-chloroperoxybenzoic acid
MOLT-4 human acute lymphoblastic leukemia cell line
MPO   multiparameter optimization
MW    molecular weight
NBS   N-bromosuccinimide
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<tr>
<td>SM</td>
<td>starting material</td>
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<tr>
<td>TEA</td>
<td>triethylamine</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>TFAA</td>
<td>trifluoroacetic anhydride</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
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<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
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<tr>
<td>TTBP</td>
<td>tri-tert-butylphosphine</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>µw</td>
<td>microwave</td>
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Chapter 1
Introduction

1.1 Neglected Diseases.

Neglected diseases represent a major challenge for the medical field. Such afflictions have no cure and limited research progress, yet still affect one billion people worldwide and kill approximately 534,000 individuals every year.\(^1\) Since neglected diseases affect the poorest areas of the world (Figure 1), major drug companies typically do not fund research on these due to low profitability.

![Figure 1. Global map of common neglected tropical diseases. Source: Molly Brady, Emory University.](image)

One method to measure the severity of a disease is through disability-adjusted life year (DALY) values. DALY values quantify the number of healthy years lost due to a disease. Therefore, the DALY value is equal to the number of years that the individual is living with the disease plus the number of years of life lost due to a premature death. A disease contracted near
the end of a patient’s life, such as prostate cancer, will have a lower DALY value than a disease that can affect a younger population, such as malaria. High DALY values can be found in sub-Saharan Africa, as shown in Figure 2.

![Figure 2](image)

**Figure 2.** Disability-adjusted life years out of 100,000 lost due to any cause in 2004. \(\square\) no data \(\square\) less than 9,250 \(\square\) 9,250–16,000 \(\square\) 16,000–22,750 \(\square\) 22,750–29,500 \(\square\) 29,500–36,250 \(\square\) 36,250–43,000 \(\square\) 43,000–49,750 \(\square\) 49,750–56,500 \(\square\) 56,500–63,250 \(\square\) 63,250–70,000 \(\square\) 70,000–80,000 \(\square\) more than 80,000 (Information from World Health Organization “Death and DALY estimates for 2004 by cause for WHO Member States,” 2004; created by Lokal_Profil).

Research and development efforts and DALY values can be compared among common diseases affecting wealthy areas and those of neglected diseases, as shown in Table 1. Prostate cancer has a total DALY value of 5.8 million and there are 80 drugs on the market for the disease. The neglected disease schistosomiasis has a comparable DALY value (4.0 million), however, no marketed drugs exist, representing the huge disproportion of research and need.
The World Health Organization (WHO) has developed a goals to fight NTDs in the 2020 Roadmap on neglected tropical diseases. The United States, United Kingdom, and United Arab Emirates governments, along with the World Bank, the Bill and Melinda Gates Foundation, and 13 pharmaceutical companies, have formed a collaboration to promote drug donation programs to increase funding of neglected diseases through the year 2020. This will provide a promising step toward better treatment for these debilitating diseases.

1.2 Human African Trypanosomiasis

Human African trypanosomiasis (HAT) is a neglected tropical disease affecting sub-Saharan Africa. The disease is caused by the parasite *Trypanosoma brucei*, which is spread by the tsetse fly. In the first stage of HAT, known as the hemolymphatic phase, trypanosomes multiply in the subcutaneous tissue, blood, and lymph nodes, causing fever, headache, itching, and joint discomfort. The second phase is the neurological phase, where the parasites cross the blood-brain
barrier, causing confusion, changes in behavior, and uncontrollable exhaustion, which is why the disease is also referred to as sleeping sickness.

There are two sub-species of the protozoan: *T. brucei gambiense*, which affects Eastern Africa, and *T. brucei rhodesiense*, which affects the Southwestern coast (Figure 3). *T. brucei gambiense* accounts for 95% of infections and leads to a chronic form of the disease. Once the infected person shows symptoms, the disease has already progressed into the central nervous system. *T. brucei rhodesiense* causes immediate symptoms and results in the more severe form of the disease; whereas symptoms are presented within weeks and the victim may be dead within months. When untreated, the disease results in coma or death due to swelling of the brain or cardiac arrest.
In 2002, WHO reported 48,000 deaths from HAT and it is estimated that 17,500 new cases arise every year. The disease is especially dangerous for pregnant women, as the fetus can also be infected. Although *T. brucei* infections occur exclusively in Africa, blood transfusions, organ transplants, drug use, and immigration have caused the infections to spread to areas such as the United States and Europe.²

Current diagnostic procedures and treatments are costly, invasive, and inefficient. To diagnose HAT, serological tests are performed to determine whether *T. brucei* is present. To diagnose the stage of the disease, a cerebrospinal fluid sample is taken through a lumbar puncture and must be made at the earliest signs of symptoms. In most areas where the disease is found, medical resources are scarce and oftentimes people will die from the disease before they are ever treated.

Treatment of HAT in the first stage includes pentamidine and suramin, shown in Figure 4. Pentamidine is used for early stage treatment of *T. brucei gambiense* infection and is injected in patients intramuscularly for ten days. Several severe side effects can occur from taking the medication, including diabetes onset, heart problems, and low blood pressure. Deaths due to hypotension or cardiac arrhythmias caused by pentamidine have been reported. Pentamidine does not cross the blood-brain barrier and can only be used for early stage trypanosomiasis. The
mechanism of action is not fully understood, however it is clear that pentamidine inhibits nuclear metabolism through DNA, RNA, phospholipid, and protein synthesis repression. Suramin, injected intravenously, treats early symptoms of *T. brucei rhodesiense* infection and inhibits protein-tyrosine phosphatases. Common side effects include stomach pain, discomfort of the skin, headache, and irritability, while less common effects include blindness, hypotension, and difficulty breathing.

Eflornithine, nifurtimox, and melarsoprol, shown in Figure 5, are treatment for the neurological stage of the disease. Eflornithine and nifurtimox treat only *T. brucei gambiense* infections. Eflornithine inhibits the *T. brucei* ornithine decarboxylase and is injected into patients. Nifurtimox, a tablet taken orally for up to four months, is only given in combination with eflornithine. Nifurtimox, taken only in combination with eflornithine, can cause anorexia in more than 10% of patients, convulsions, depression, and headache.

Melarsoprol is derived from arsenic and is very toxic, causing fatality in 3-10% of patients. The drug inhibits pyruvate kinase in the trypanosome, thus diminishing glycolysis and generation of ATP and killing the parasite. Reactive arsenical encephalopathy develops in 10-12% of patients taking melarsoprol. Injections of melarsoprol, which are taken for ten days, are extremely painful for patients. It is used even though it is extremely toxic because it is the only drug available for second stage infections by *T. brucei rhodesiense*.

![Figure 5. HAT treatment for the neurological phase.](image-url)
More efficient and cost-effective treatment is needed for HAT. All current drugs require invasive delivery, which is especially impractical for the afflicted population living in rural Africa. Vaccination against *T. brucei* is not an option. The parasite has variant surface glycoproteins on the outside of the cells that allow it to undergo antigenic variation, which causes the glycoprotein coat to frequently change and evade the immune system. The immune system response on the parasite is thus ineffective and vaccines are useless since the target constantly changes. Prevention can only occur by vector control; however, since *Silent Spring* was published, which discussed the huge environmental impact from killing a species, large scale pesticide distribution is not advised. Drug discovery efforts are currently underway to develop a compound that effectively inhibits the parasite with little effect on human cells that can ideally be administered orally for convenient therapy.

### 1.3 Target Repurposing

Two types of drug discovery approaches exist: phenotypic-based and target-based. With phenotypic-based drug discovery, compounds are tested against whole cells or organisms to see if the desired phenotype (such as growth inhibition, cell cycle arrest, etc) is achieved. For example, a large library of compounds are screened against the parasite and compounds that show inhibition are then optimized. With target-based drug discovery, compounds are designed based on a specific enzyme or receptor target that has been implicated in a disease state.

Kinases, enzymes that assist with phosphorylation, are the largest group of targets in target-based drug discovery for humans, making up of 22% of the “Druggable Genome.” Since the Trypanosomatid kinome is similar to that in humans, several approaches to target *T. brucei* kinases in the effort to inhibit the parasite have been made. Researchers have found a correlation between
glycogen synthase kinase-3 short, phosphofructokinase, phosphoglycerate kinase, and hexokinase inhibition and *T. brucei* inhibition.

Another approach based on target-based drug discovery is target repurposing. This method shortens the total drug development process by starting with an established target inhibitor and altering the function to selectively inhibit the parasitic target. For example, danusertib is an Aurora kinase inhibitor in Phase II clinical trials for solid tumor therapy. Since three Aurora kinases are expressed in *T. brucei*, danusertib was screened against *T. brucei rhodesiense* and was found to inhibit the parasite with an IC$_{50}$ of 0.15 µM. From these promising results, 19 analogs were synthesized in an attempt to increase potency and selectivity over human cells. While NEU327 was slightly less potent toward the parasite (IC$_{50}$ = 0.61 µM), it was 7-fold selective over the human cells (Figure 6), confirming that a human kinase inhibitor can be repurposed as a starting point to discover compounds that can selectively inhibit parasite growth.

Use of an established tyrosine kinase inhibitor (tyrphostin) led to inhibition of transferrin uptake, which inhibited parasite growth. Based on these results, lapatinib, a receptor tyrosine kinase inhibitor currently used clinically for treatment of breast cancer, was used as a starting

![Figure 6. Analog designed from danusertib to selectively inhibit *T. brucei*.](image)
compound for evaluating *T. brucei* inhibition. Lapatinib was screened against *T. brucei* and HepG2 cells, giving IC\(_{50}\)s of 1.54 µM and 6.2 µM, respectively. Starting from the lapatinib structure, 44 analogs were made in the attempts to increase potency and selectivity.\(^\text{18}\) **NEU617** was 37-fold more potent than lapatinib with an IC\(_{50}\) of 0.042 µM and had excellent selectivity (Figure 7), reconfirming target-repurposing as a rapid lead discovery approach.

![Figure 7. Analog designed from lapatinib to selectively inhibit *T. brucei*.](image)

### 1.4 High Throughput Screen.

Since several kinases have been shown to be an effective target for *T. brucei* inhibition, a high throughput screen (HTS) tested 42,444 compounds from a kinase inhibitor library at GlaxoSmithKline against *T. brucei* in a whole cell assay. The compounds were screened at 4 µM and those with over 50% inhibition were selected (Figure 8). Of the resulting 4,574 compounds, the IC\(_{50}\)s against *T. brucei* and HepG2 cells were measured. Compounds with IC\(_{50}\)s of less than 1 µM and over 100 fold selectivity were chosen, resulting in 797 compounds. These compounds were clustered and the physicochemical properties were calculated, leading to 59 clusters and 53 singletons.\(^\text{19}\)
Clusters 20-22 were chosen for further evaluation due to the large number of compounds in the cluster (n = 57), the high potency of many of the compounds, and the chemical similarity of the compounds. Of the 57 compounds in the clusters, the chemical compositions of ten were released by GSK, as shown in Figure 9. Clusters 20-22 were grouped together due to the similarities of the structures. Nearly all of the compounds in this grouping contain an azaindole “head” group, except for GSK1560285A. The given structures all had excellent potency from sub-nanomolar (IC\textsubscript{50} = 0.8 nM) to sub-micromolar (IC\textsubscript{50} = 339 nM) range.
**Figure 9.** Structures given of HTS hits in clusters 20-22 and the corresponding *T. brucei* IC\textsubscript{50} values.
2.1 Medicinal Chemistry Approach 1.

To explore the chemical diversity, a structure activity relationship was performed on the cluster. For ease of synthesis, the phenyl core of compound GSK1873475A was chosen to for the first analogs. To determine the role of the halogen and the importance of the stereocenter, the racemic analog NEU-1052 was proposed based on HTS hit GSK1873475A, as shown in Scheme 1.

To synthesize NEU-1052, the azaindole head group can be attached to the lower region of the compound via Suzuki coupling, as shown in Scheme 2. Since either fragment can be converted to the boronic ester from the
aromatic halide, the best results were determined through experimental efforts. Commercially available 4-bromobenzoic acid and 2-phenyl glycinol can be combined to give the southern intermediate while azaindole can be the starting point for the northern intermediate.

2.1.1 Chemistry of Analog 1.

Based on the retrosynthetic plan described above, efforts to prepare key intermediates 2 and 4 commenced as shown in Scheme 3. DL-α-phenylglycine was reduced to the corresponding alcohol (2) using previously reported conditions utilizing sodium borohydride and iodine,\(^{21}\) giving a quantitative yield (Scheme 3). The product was then coupled to 4-bromobenzoic acid using EDC coupling to give intermediate 2 in 61% yield.

![Scheme 3](image)

**Scheme 3.** Reaction conditions: (a) i. NaBH\(_4\), I\(_2\), THF, 64 °C; ii. MeOH, 100%; (b) EDC, HOBt, DIPEA, DMF, rt, 61%; (c) NBS, H\(_2\)SO\(_4\), THF, rt, 53%; (d) TsCl, TEA, DMAP, DCM, 80 °C to rt, 71%

Subsequently, 7-azaindole was brominated at the 3 position with previously reported conditions using NBS\(^{22}\) to give 3 in 53% yield. Compound 3 was tosylated using \(p\)-toluenesulfonyl chloride, triethylamine, and DMAP to give 4 in 71% yield.

To couple intermediates 2 and 4 using Suzuki conditions,\(^{20}\) one brominated compound must be converted to the corresponding boronic ester or boronic acid, as shown in Scheme 4. Borylation of compound 3, shown in Scheme 4, was first attempted using bis(pinacolato)diboron,
Pd(dppf)Cl₂, and potassium acetate. Several different conditions were also attempted, including screening solvents (dioxane, THF, and DMF), trying a different catalyst (Pd₂(dba)₃ with tricyclohexylphosphine), and attempting conventional and microwave conditions, as shown in Table 2. For all entries, only low conversion rates were obtained and the product could not be separated via chromatography. Borylation of the tosyl protected intermediate 4 was also

<table>
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<th>Catalyst</th>
<th>Additive</th>
<th>Solvent</th>
<th>Temp</th>
<th>Result</th>
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<td>3</td>
<td>1.1</td>
<td>Pd(dppf)Cl₂</td>
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<td>dioxane</td>
<td>µw, 145 °C</td>
<td>Impure product obtained</td>
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<td>2</td>
<td>3</td>
<td>1.1</td>
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<td>-</td>
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<td>Reflux</td>
<td>No product conversion</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>1.1</td>
<td>Pd(dppf)Cl₂</td>
<td>-</td>
<td>DMF</td>
<td>95 °C</td>
<td>No product conversion</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>1.5</td>
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<td>-</td>
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<tr>
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<td>3</td>
<td>1.2</td>
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<td>P(Cy)₃</td>
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<td>Product plus SM</td>
</tr>
<tr>
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<td>4</td>
<td>1.1</td>
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<td>-</td>
<td>dioxane</td>
<td>µw, 145 °C</td>
<td>Impure product obtained</td>
</tr>
</tbody>
</table>

Table 2. Borylation reagents screen.
attempted, however, similar results to that of 3 were observed (Table 2). Due to these poor results, borylation of the other brominated intermediate (2) was attempted.

The boronic ester of intermediate 2 was successfully synthesized using bis(pinacolato)diboron, Pd(dppf)Cl₂, and potassium acetate in DMF to give 5 in 91% yield. Compound 5 was coupled with 4 using Pd(PPh₃)₄ and sodium carbonate to give NEU-1051 in 36% yield. NEU-1051 was deprotected using NaOH in dioxane to give the desired analog (NEU-1052) in 45% yield (Scheme 4).

2.1.2 Biological Screen and Discussion 1.

Compound NEU-1052 and the tosyl protected corresponding analog NEU-1051 were screened against T. brucei, giving EC₅₀s of 0.787 µM and >3 µM, respectively. These results show that the tosyl group hinders inhibition, suggesting that the indole NH may be involved in active site binding. The seven fold decrease in potency of NEU-1052 over GSK1873475A (EC₅₀ of 0.787 versus 0.135 µM) indicates that either the fluorine at the 4 position of the azaindole or the R stereocenter increases potency, or both.

2.2 Medicinal Chemistry Approach 2.

The compounds in clusters 20-22 were reevaluated in an alternative medicinal chemistry approach to improve the physicochemical properties while also exploring SAR. In an effort to improve the drug-like properties of the series, the central nervous system multiparameter optimization score (CNS MPO) was used.²³ The CNS MPO scoring is a method used to predict penetration in the brain based on the calculated physicochemical properties of the compound. The guidelines were determined by evaluating the ClogP, ClogD, molecular weight, topological polar
surface area, number of hydrogen bond donors, and \( pK_a \) of 119 CNS drugs on the market including 108 Pfizer CNS drug candidates. An algorithmic scoring system was developed based on the probability of CNS drugs on the market having specific physicochemical criteria, and optimal ranges were created. The scoring system is designed on the basis that if one physicochemical property is not ideal, the compound will not be discarded as a possible CNS candidate. Each property is scored from zero (unfavorable CNS property) to one (favorable CNS property), and these property scores are summed. The six properties of the marketed CNS drugs were scored and the most likely score was \( \geq 4 \), as shown in Figure 10.

<table>
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<th>Properties</th>
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<th>Less desirable range (score of 0)</th>
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<td>ClogP &gt; 5</td>
</tr>
<tr>
<td>ClogD</td>
<td>ClogD \leq 2</td>
<td>ClogD &gt; 4</td>
</tr>
<tr>
<td>MW</td>
<td>MW \leq 360</td>
<td>MW &gt; 500</td>
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<tr>
<td>TPSA</td>
<td>40 &lt; TPSA \leq 90</td>
<td>TPSA \leq 20; TPSA &gt; 120</td>
</tr>
<tr>
<td>BD</td>
<td>HBD \leq 0.5</td>
<td>HBD &gt; 3.5</td>
</tr>
<tr>
<td>( pK_a )</td>
<td>( pK_a \leq 8 )</td>
<td>( pK_a &gt; 10 )</td>
</tr>
</tbody>
</table>

The MPO scores of the ten HTS hits were calculated (Table 3) using physicochemical properties measured using JChem for Excel. Compound GSK1081046A was the most potent with subnanomolar potency (IC\(_{50}\) = 0.8 nM); however, the
physicochemical properties were undesirable for brain penetration. GSK1873472A, containing the furan core, had the best MPO score with a value of 4.0.

In an effort to mimic the regiochemistry of GSK1081046A while maintaining similar physicochemical properties of GSK1873472A, analog NEU-2139 was designed, as shown in Figure 11.

To synthesize NEU-2139, the azaindole-furan intermediate can be coupled to the amino fragment via several different methods. The azaindole-furan (6) can be aminated and coupled to the amino acid (7) via conditions such as EDC coupling, or brominated and coupled to the corresponding amide under Buchwald or CuI

<table>
<thead>
<tr>
<th>HTS Hit</th>
<th>ClogP</th>
<th>ClogD</th>
<th>TPSA</th>
<th>MW</th>
<th>HBD</th>
<th>pKa</th>
<th>MPO score</th>
</tr>
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<tr>
<td>GSK1229959A</td>
<td>2.84</td>
<td>1.12</td>
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<td>434</td>
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<td>13.7</td>
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<td>GSK1873475A</td>
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<td>3.18</td>
<td>78</td>
<td>375</td>
<td>3</td>
<td>13.3</td>
<td>3.4</td>
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<td>GSK1282963A</td>
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<td>0.376</td>
<td>98.3</td>
<td>435</td>
<td>3</td>
<td>12.8</td>
<td>3.4</td>
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<tr>
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<td>2.24</td>
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<td>3</td>
<td>11.8</td>
<td>4.0</td>
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<tr>
<td>GSK1657594A</td>
<td>2.79</td>
<td>2.79</td>
<td>87.2</td>
<td>507</td>
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<tr>
<td>GSK1329819A</td>
<td>2.98</td>
<td>0.703</td>
<td>85.4</td>
<td>448</td>
<td>3</td>
<td>11.1</td>
<td>3.5</td>
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<td>GSK1560285A</td>
<td>1.8</td>
<td>-0.573</td>
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<td>13.4</td>
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<td>GSK1669841A</td>
<td>3.39</td>
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<td>87.2</td>
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<td>2.3</td>
</tr>
<tr>
<td>GSK1580292A</td>
<td>2.77</td>
<td>0.940</td>
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<td>GSK1081046A</td>
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<td>2.01</td>
<td>74.4</td>
<td>416</td>
<td>3</td>
<td>11.0</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Table 3. Calculated physicochemical properties and CNS MPO scores of HTS hits.

Figure 11. Design of analog NEU-2139 from HTS hits.
coupling conditions (Scheme 5).

To give the azaindole-furan intermediate 6, the boronic acid of either the azaindole (8) or the furan (9) can be combined with the halogenated counterpart.

2.2.1 Chemistry of Analog 2.

The second analog was synthesized by oxidizing 7-azaindole using previously reported conditions\(^2\) to give 10 as a \textit{m}-chlorobenzoate salt in 90\% yield (Scheme 6). Compound 10 was chlorinated at the 4 position with reported conditions\(^3\) resulting in 11 in 75\% yield. The synthesized 4-chloro-7-azaindole was then tosylated using TsCl, TEA, and DMAP to give 12 in 93\% yield.

![Scheme 5. Retrosynthesis of analog NEU-2139.](image)

From 12, several different approaches were attempted, as shown in Scheme 7. Each approach will be discussed in the following sub-sections. In the first synthetic route (Section 2.2.2), the furan core was coupled to 12 using 2-furanylboronic acid. From there, both nitration (Section 2.2.3) and bromination followed by amination (Section 2.2.5) were attempted at the 5
position. In addition, a Curtius Rearrangement was attempted using 5-formyl-2-furanyl boronic acid (Section 2.2.4). Borylation of 12 followed by attachment of the brominated furan was attempted as will be discussed in Section 2.2.6. Finally, the amino acid phenylalanine was converted to the corresponding amine and coupled to the brominated intermediate (Section 2.2.7).

2.2.2 Attachment of the Furanyl Core.

Compound 12 was coupled with 2-furanylboronic acid using conditions previously utilized to synthesize NEU-1051 (See Section 2.1.1). This procedure using Pd(PPh₃)₄ gave 13 in a
maximum yield of 45% (Scheme 8). To improve the yield, a different procedure was applied.\textsuperscript{26} Using Pd\textsubscript{2}(dba)\textsubscript{3}, tri-\textit{tert}-butylphosphine, and potassium fluoride, the yield was improved to 85%.

2.2.3 Attempt to Nitrate the Furanyl Core.

Nitration was attempted on compound 13, using various procedures including TFAA and nitric acid,\textsuperscript{27} acetic anhydride and nitric acid,\textsuperscript{28} and oxalylchloride, DMF, and sodium nitrate.\textsuperscript{29} All procedures gave low conversion rates or byproducts with different nitrated positions (Table 4). Acetic anhydride and nitric acid provided the only separable product (14), albeit in very low (9%) yield (Scheme 9).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagents</th>
<th>Temperature</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>nitric acid, TFAA</td>
<td>-15 °C</td>
<td>No reaction, SM unable to dissolve in TFAA</td>
</tr>
<tr>
<td>2</td>
<td>sodium nitrate, oxalyl chloride, DMF</td>
<td>rt</td>
<td>SM + side products</td>
</tr>
<tr>
<td>3</td>
<td>nitric acid, Ac\textsubscript{2}O</td>
<td>-30 °C to rt</td>
<td>Pdt + other nitrated position, inseparable</td>
</tr>
<tr>
<td>4</td>
<td>nitric acid, Ac\textsubscript{2}O</td>
<td>-30 °C</td>
<td>Product in 9% yield</td>
</tr>
</tbody>
</table>

Table 4. Nitrination attempts.

2.2.4 Curtius Rearrangement Attempt.

A different approach to amine formation was attempted via a Curtius rearrangement strategy, which would generate the Boc-protected amine (17). Several different conditions were
attempted to couple compound 12 with 5-formyl-2-furanylboronic acid (Scheme 10). Pd(PPh₃)₄ and potassium carbonate in dioxane did not lead to any product formation, while PdCl₂(PPh₃)₂ and sodium carbonate in DME/water resulted in trace product. Pd₂(dba)₃ with tri-tert-butylphosphine and KF in dioxane gave the best results leading to formation of 15 in 60% yield (Table 5). To oxidize the aldehyde to the corresponding carboxylic acid (16), sodium chlorite in water and acetonitrile was used, however, the reaction led to no product formation. Copper chloride and t-butyl hydroperoxide in acetonitrile gave partial conversion. Subsequently, diphenylphosphoryl azide was synthesized from diphenylphosphochloridate and sodium azide in acetone giving a 43% yield. Due to the increased number of steps and low yields, this synthetic route was abandoned.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Additive</th>
<th>Base</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pd(PPh₃)₄</td>
<td>-</td>
<td>K₂CO₃</td>
<td>Dioxane</td>
<td>100 °C</td>
<td>No product conversion</td>
</tr>
<tr>
<td>2</td>
<td>PdCl₂(PPh₃)₂</td>
<td>-</td>
<td>Na₂CO₃</td>
<td>EtOH/DME</td>
<td>50 °C</td>
<td>5% conversion, 95% SM</td>
</tr>
<tr>
<td>3</td>
<td>Pd₂(dba)₃</td>
<td>TTBP</td>
<td>KF</td>
<td>Dioxane</td>
<td>100 °C</td>
<td>Product in 60% yield</td>
</tr>
</tbody>
</table>

Table 5. Conditions attempted to couple 5-formyl-2-furanylboronic acid to 12.

2.2.5 Bromination of the furanyl core followed by attempts to aminate.
By brominating 13, the complex can be converted to the amine using several different methods. Bromination of compound 13 was attempted at the 5 position with NBS to give 18 (Scheme 11). The reaction had low yields (25%) due to the simultaneous bromination of the benzylic methyl on the tosylamide moiety. Direct bromination was then attempted, and this reaction showed good conversion via LC-MS and TLC. However, most of the product was lost during workup. It was discovered that any remaining bromine created byproducts and HBr salts during the reaction workup, which entailed concentration of the solution by rotary evaporation. Instead of using the rotary evaporator to remove the bromine, the solid was filtered after completion of the reaction. Residual bromination proved problematic, as methanol washes of the filtered solid was also found to produce unwanted byproducts. In response to this, any remaining bromine was removed by dissolving the filtered solid in DCM and washing with 10% sodium thiosulfate until the orange color disappeared. This optimized workup procedure increased the yield from 25% to 60%.

The conversion of 18 to the corresponding amine (19) was attempted through various routes, as shown in Scheme 12. Direct conversion was attempted by using lithium bis(trimethylsilyl)amide, Pd₂(dba)₃, and (2-biphenyl)dicyclohexylphosphine in THF. Only the N-deprotected starting material was formed. Compound 18 was also stirred in ammonium hydroxide, however, only trace amount of product was formed.

Instead of directly forming the primary amine, preparation of the corresponding benzyl amine (20) was attempted. Compound 18, benzyl amine, and pyridine led to no product formation as well. The phthalimide formation to give 21 was attempted using freshly synthesized potassium
phthalimide and copper iodide in DMA, however, no desired product was observed, with either conventional or microwave heating. Synthesis of the Boc-protected-amine (17) from 18 was attempted using Buchwald conditions, reacting 18 with tert-butyl carbonate in the presence of Pd$_2$(dba)$_3$, di-tert-butyl(2’,4’,6’-triisopropyl-[1,1’-biphenyl]-2-yl)phosphine, and cesium carbonate in t-butanol and dioxane. No product was observed. Alternatively, a reaction was attempted using Pd(OAc)$_2$, palladium acetate, XantPhos, cesium carbonate, and tertbutylcarbamate in dioxane, giving 17 in 18% yield. After initial attempts to deprotect 17 using both TFA and HCl gave no product, it was concluded that 17 may be impure. Due to the low yield and failure of the next step, this synthetic route was abandoned.

### 2.2.6 Boronic ester formation followed by furan coupling.

Since the amine formation from 18 gave poor results, access to the desired product was attempted by aminating the furan before the azaindole coupling in an effort to form 17, as presented in Scheme 13. Borylation of 12 was attempted along with the parallel synthesis of the amino
bromofuran (24, Scheme 14). Borylation of 12 was attempted using n-butyllithium and triisopropyl borate in THF, however, no reaction occurred. The boronic ester was synthesized by reacting 12 with bis(pinacolato)diboron, Pd$_2$(dba)$_3$, XantPhos, and potassium acetate in dioxane. Due to two major side product formation, compound 22 was synthesized in low yield (10%).

Dibromination of furan (23) followed by amination (24), as shown in Scheme 14, was attempted in an effort to couple with 22 (Scheme 13). Dibromination using both NBS and bromine was attempted. NBS gave no product while bromine gave only 1% yield, probably due to loss of product during distillation. Chromatography could not be used perhaps due to high volatility, and steam distillation required large scale and resulted in no pure product. Due to poor results of both the borylation of azaindole and furan bromination, a different approach was taken.

2.2.7 Amide formation followed by CuI coupling.
The amino acids were converted into the corresponding amide (Scheme 15) in attempts to couple to 18. L-phenylalanine was Boc-protected using Boc-anhydride and sodium carbonate, giving 25 in quantitative yield. Compound 25 was then converted to the amide using ethyl chloroformate, triethylamine, and ammonium hydroxide to give 26 in 78% yield.

Coupling the amide 26 to the brominated furan-azaindole complex 18 was attempted using several different procedures (Table 6). Buchwald conditions using palladium(II) acetate, XantPhos, and cesium carbonate in dioxane were first attempted. Trace amount of conversion was detected by LC-MS, however, the main byproduct was 27, as shown in Scheme 16, in which the azaindole-furan complex was substituted twice with the amide protons. When switching the catalyst to Pd₂(dba)₃ and heating with microwave conditions, more side products were formed.

The coupling of 18 with amide 26 was then attempted through copper iodide coupling in an Ullmann-type reaction as shown in Table 6. Copper iodide, N,N’-dimethylethylenediamine, and potassium carbonate in degased dioxane at 110 °C gave mostly reduced starting material (13)
along with side product 27 and a small amount of product conversion. In an attempt to reduce production of 13, the temperature was lowered to 100 °C, however, no improved product conversion was observed. An excess of the amide 26 was used to prevent multiple addition of the furan-azaindole complex to the nucleophile. Two equivalents of 26 lead to little improvement while three equivalents lead to little to no product formation. The most promising results were with 1.5 equivalents of 26. The base was switched to potassium phosphate which lead to less product formation. Similarly, the amine ligand trans-N,N'-dimethylcyclohexyl-1,2-diamine was used in place of N,N'-dimethylethylene diamine, however, the change also lead to less product conversion. The best conditions were then tried with molecular sieves and freshly distilled diamine, leading to isolable product **NEU-2126**, shown in **Scheme 17**, in 25% yield. Dropwise addition of 18 in dioxane after all reactions were added to the vial lead to an improved yield of 32%. By reproducing the reaction several times, it was noticed that yield decreases with increasing

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Additive</th>
<th>Base</th>
<th>Temperature, time</th>
<th>Comment</th>
<th>Results</th>
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</thead>
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<td>Pd(OAc)$_2$</td>
<td>XantPhos</td>
<td>Cs$_2$CO$_3$</td>
<td>120 °C, 1h</td>
<td>-</td>
<td>80% 27 conversion by LC</td>
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<tr>
<td>2</td>
<td>Pd$_2$(dba)$_3$</td>
<td>XantPhos</td>
<td>Cs$_2$CO$_3$</td>
<td>150 °C, µW, 30 min</td>
<td>-</td>
<td>10% product conversion, many other side products observed by LC</td>
</tr>
<tr>
<td>3</td>
<td>CuI</td>
<td>CH$_3$NH(CH$_2$)$_2$NHCH$_3$</td>
<td>K$_2$CO$_3$</td>
<td>110 °C, overnight</td>
<td>-</td>
<td>13 is main peak by LC</td>
</tr>
<tr>
<td>4</td>
<td>CuI</td>
<td>CH$_3$NH(CH$_2$)$_2$NHCH$_3$</td>
<td>K$_2$CO$_3$</td>
<td>100 °C, 3h</td>
<td>-</td>
<td>1:1:1:1 ratio of 18, 13, 27, product</td>
</tr>
<tr>
<td>5</td>
<td>CuI</td>
<td>trans-N,N'-dimethylcyclohexyl-1,2-diamine</td>
<td>K$_2$CO$_3$</td>
<td>101 °C, 1h</td>
<td>-</td>
<td>Mostly 13 and 27</td>
</tr>
<tr>
<td>6</td>
<td>CuI</td>
<td>CH$_3$NH(CH$_2$)$_2$NHCH$_3$</td>
<td>K$_2$CO$_3$</td>
<td>110 °C, overnight</td>
<td>3 eq. 26</td>
<td>Mostly 13 and 27</td>
</tr>
<tr>
<td>7</td>
<td>CuI</td>
<td>CH$_3$NH(CH$_2$)$_2$NHCH$_3$</td>
<td>K$_3$PO$_4$</td>
<td>110 °C, overnight</td>
<td>-</td>
<td>Mostly 13 and 27</td>
</tr>
<tr>
<td>9</td>
<td>CuI</td>
<td>CH$_3$NH(CH$_2$)$_2$NHCH$_3$</td>
<td>K$_2$CO$_3$</td>
<td>101 °C, overnight</td>
<td>1.5 eq. 26, 18 added dropwise</td>
<td>Product in 32% yield</td>
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</table>

**Table 6.** Coupling attempts of 18 to 26.
lifespan of the anhydrous solvent and therefore, only freshly distilled dioxane should be used for the reaction.

Compound **NEU-2126** was Boc-deprotected using HCl in dioxane and methanol to give **NEU-2127** in 40% yield. When repeating the reaction with only HCl and dioxane, it was noticed that methanol is crucial for the reaction to proceed. The tosyl group of **NEU-2127** was removed using aqueous NaOH in methanol to give the final analog **NEU-2139** (Scheme 17).

![Scheme 17](image)

**Scheme 17.** Reaction conditions: (a) 26, Cul, K₂CO₃, MeNH(CH₂)₂NHMe, dioxane, 110 °C, 32%; (b) HCl/dioxane, MeOH, rt, 4h, 56%; (c) aq. NaOH, MeOH, 55 °C, 30 min, 32%

### 2.2.8 Biological Screen and Discussion

**NEU-2126** and **NEU-2127** were screened against *T. brucei*. **NEU-2126** had an IC₅₀ of 1.01 µM, a value 31-fold less potent than the HTS compounds **GSK1873472A** (IC₅₀=32 nM) and over 1000-fold less potent than **GSK1081046A** (IC₅₀=0.8 nM). Surprisingly, **NEU-2127** was also less potent, with an IC₅₀ of 1.99 µM. While it was hypothesized that the NH₂ would improve activity (consistent with the HTS data), this was not borne out by experiment. The biological data for **NEU-2139** is currently pending.
2.3 Future work.

2.3.1 Proposal of Additional Analogs.

The brominated furan-azaindole complex, (18), previously synthesized (See Section 2.2.1), can be coupled to many other amides. In an effort to test the structure activity relationship and to improve the CNS MPO score, several amino acids that can be converted to the corresponding amide, were identified (Figure 12). Amino acid 28 was proposed because it has the same regiochemistry as the HTS hit GSK1081046A while reducing the molecular weight. Proposed amino acid 29 has the same regiochemistry as the synthesized amino acid 25 without the lipophilic benzyl ring. Amino acid 30 is the same as that in GSK1081046A which will allow to directly compare the different cores. Amino acid 31 eliminates the benzyl group which will both reduce lipophilicity and decrease the molecular weight. Amino acids 32 and 33 reduce the size of the ring which will also decrease the molecular weight and potentially the lipophilicity.

In addition to coupling to 18, the amino acids can also be coupled to different cores, since the previously attempted CuI coupling of 18 to the amide (26) gave inconsistent results with poor yields. Based on the most synthetically accessible compounds, the four cores shown in Figure 13...
were proposed. Each core contains amine functionality which should result in more facile coupling to the amino acid, compared to an amindation from the aryl bromide (ie 18).

Cores 35 – 37 can be synthesized via the 7-azaindole-4-carboxylic acid intermediate, as shown in Scheme 18. Proposed core 34 can be synthesized from the corresponding nitro complex (38), which can be composed by coupling 7-azaindole-4-boronic acid to 5-bromo-2-nitro-thiophene (Scheme 19).
2.3.2 Progress toward amino acid and amide synthesis.

Synthesis of the proposed amino acids 28 – 33 is currently underway. The Boc-protected amino acid 39 was purchased and converted into the corresponding amide using oxalyl chloride and triethylamine, followed by ammonium hydroxide, in 73% yield, as shown in Scheme 20. Compound 40 can then be coupled to the azaindole-furan core 18 to directly compare biological activity with that of synthesized analog NEU-2139.

Amino acid piperidine-3-carboxylic acid (31) was purchased and converted to the Boc-protected amino acids 41 and 45, as depicted in Scheme 21. Compound 31 was Boc-protected with previously reported conditions using Boc-anhydride and sodium carbonate to give 41 in 86% yield.

To obtain 45, compound 31 was first converted to the corresponding ethyl ester in a Fisher esterification reaction using thionyl chloride and ethanol, giving 42 in 97% yield. Compound 42

\[ \text{Scheme 20. Reaction conditions: (a) i. oxalyl chloride, TEA, 0°C, 30 min; ii. NH}_4\text{OH, rt, 1h, 73%} \]

\[ \text{Scheme 21. Reaction conditions: (a) Boc}_2\text{O, Na}_2\text{CO}_3, \text{THF/water, 0°C to rt, 24h, 86%; (b) SOCl}_2, \text{EtOH, 0°C to reflux, 5h, 97%; (c) Boc}_2\text{O, TEA, DCM, rt, 16h, 88%; (d) LDA, BnBr, THF, -78°C to rt, 2d, 59%; (e) aq. NaOH, EtOH, reflux, 1d, 84%} \]
was protected using Boc-anhydride and triethylamine to give 43 in 88% yield. The benzyl substituent was added to the 3-position using lithium diisopropylamine and benzyl bromide to give 44 in 59% yield. From 44, hydrolysis of the ester using sodium hydroxide gave 45 in 84% yield.

Amino acids 28 and 52 can be synthesized from diethyl benzylmalonate, as shown in Scheme 22. To synthesize 28, first, N-(bromomethyl)phthalamide (47) was synthesized. From phthalimide, the methylhydroxy substituent was added using formaldehyde to give 46 in 96% yield. Using HBr and sulfuric acid, 47 was produced in 72% yield. To diethyl benzylmalonate was added sodium hydride and 47 to give 48 in 82% yield. The free amine can be synthesized from 48 using HCl and acetic acid to give 28 (reaction not yet completed).

Scheme 22. Reaction conditions: (a) formaldehyde, water, 100 °C, 4h, 96%; (b) HBr, H₂SO₄, 65 °C, 5h, 72%; (c) NaH, THF, 16h, 82%; (d) HCl, acetic acid, 120 °C, 2d; (e) KOH, EtOH, rt, 16h, 80%; (f) formaldehyde, water, DEA, 6h, rt, 65%; (g) TFA, rt, 3h, 34%; (h) NaOH, MeOH, reflux, 16h
To synthesize the benzyl protected amino acid 32 (compound 52), diethyl benzylmalonate was partially hydrolyzed using potassium hydroxide to give 49 in 66% yield. In a combined aldol condensation-decarboxylation reaction, 50 was synthesized from 49 using formaldehyde and DEA to give product in 65% yield. From 50, TFA and N-(methoxymethyl)-N-(trimethylsilylmethyl)benzylamine gave 51 in 34% yield. Sodium hydroxide can be used to obtain 52 (product not yet obtained).

**Chapter 3 Conclusion**

3.1 Conclusion.

Several analogs were designed from HTS data to explore SAR, improve physicochemical properties, and maintain potency against *T. brucei*. Use of a CNS MPO scoring system allows for a quantitative method of guiding analog designs with appropriate drug-like properties. While NEU-1052 was not more potent than the corresponding hit compound, it was more potent than NEU-1051, suggesting that the azaindole NH promotes parasite inhibition. Additional compounds are pending data (NEU-2126 and NEU-2127), and additional analogs with promising predicted physicochemical properties have been proposed for future study.
Chapter 4 Experimental Procedures

4.1 General Methods.

Reagents were purchased from common suppliers including Sigma-Aldrich, Inc. (St. Louis, MO), Fisher Scientific, Frontier Scientific Services, Inc. (Newark, DE), and Matrix Scientific (Columbia, SC) and were used as received unless otherwise noted. All non-aqueous reactions were performed under nitrogen. THF, DMF, and DCM were dried and purified by passage through activated alumina columns on a purification system manufactured by Innovative Technology (Newburyport, MA). Microwave reactions were run using Biotage® Initiator Eight automated microwave in normal mode. Silica gel chromatography was performed on 230-400 mesh silica gel (SilicaFlash®) using glass columns, or with use of a Biotage® Isolera™One flash purification system. LC-MS analysis was conducted using a Waters Micromass ZQ mass spectrometer in positive mode with 254 nm UV detection by a Waters 2489 UV/visible detector. HPLC analyses were performed using a SunFire™ C18 column (4.6 mm x 50 mm, 3.5 μm column; 10 μL injection; 5% to 100% acetonitrile/water & 0.1% formic acid gradient; 150 mL/min flow). Preparative LC-MS was performed on a Waters FractionLynx system with a Waters Micro Mass ZQ mass spectrometer (electrospray ionization) and a single-wavelength UV-visible detector, using acetonitrile/H₂O gradients with 0.1% formic acid as a modifier, unless noted otherwise. ¹H NMR were recorded on a Varian spectrometer (400 MHz or 500 MHz) at room temperature. Reported chemical shifts (δ) are given in parts per million (ppm) and are referenced to residual proton solvent signals. Data for ¹H NMR spectra is formatted as follows: δ ppm, multiplicity (s for singlet, d for doublet, t for triplet, dd for doublet of doublets, m for multiplet), coupling constant (Hz), integration.
4.2 Experimental Details.

2-amino-2-phenylethanol (1). 2-amino-2-phenylacetic acid (100 mg, 0.662 mmol) and NaBH$_4$ (62.6 mg, 1.65 mmol) in anhydrous THF were cooled to 0 °C. Iodine (201 mg, 0.794 mmol) in 1 mL THF was added dropwise to the solution which was then heated to 64 °C for 24 hours under nitrogen using a reflux condenser. MeOH was added to the solution until it turned clear (about 8 drops), and the solution was stirred for 30 minutes. To this was added 2 mL of 20% KOH and the solution was stirred for 4 hours followed by extraction with DCM. Following evaporation, 91 mg of product were collected, representing quantitative yield. $^1$H (400 MHz, CDCl$_3$) δ ppm 3.52-3.56 (t, 1H), 3.72-3.75 (dd, $J$=11, 4.4 Hz, 1H), 4.02-4.05 (dd, $J$=8.1, 4.4 Hz, 1H), 7.27-7.37 (m, 5H).

4-bromo-N-(2-hydroxy-1-phenylethyl)benzamide (2). 4-bromobenzoic acid (31.2 mg, 0.155 mmol), EDC (32.7 mg, 0.171 mmol), HOBT (26.2 mg, 0.171 mmol), and DIPEA (54.2 µl, 0.311 mmol) in DMF were stirred for 20 min then added to a vial containing the prepared oil 2-amino-2-phenylethanol (21.3 mg, 0.155 mmol). Saturated aqueous sodium bicarbonate was added and the reaction was extracted with ethyl acetate. The organic layer was washed with brine, dried
with magnesium sulfate, filtered, and the solvent removed. The product was separated by chromatography (1:1 ethyl acetate to hexane) to give 15 mg solid in 61% yield. TLC of product stained with ninhydrin resulting in no color to confirm the alcohol product was produced rather than the amine. \(^1\)H (400 MHz, CDCl\(_3\)) \(\delta\) ppm 4.04 (d, \(J=4.4\) Hz, 2H), 5.26-5.30 (m, 1H), 6.80-6.84 (m, 1H), 7.34-7.42 (m, 5H), 7.56 (d, \(J=8.8\) Hz, 2H), 7.69 (d, \(J=8.8\) Hz, 2H). LCMS found 320.0, 322.0 [M + H]\(^+\).

3-bromo-1H-pyrrolo[2,3-b]pyridine (3). To 1H-pyrrolo[2,3-b]pyridine (207 mg, 1.75 mmol) in THF, 1-bromopyrrolidine-2,5-dione (331 mg, 1.86 mmol) was added under nitrogen. The solution went from clear to pale yellow. To the reaction, one drop of con. H\(_2\)SO\(_4\) (93 \(\mu\)l, 1.78 mmol) was added and was stirred at room temperature for two days. The reaction turned cloudy yellow after addition of sulfuric acid. Saturated ammonium chloride was added and the aqueous layer was extracted with ethyl acetate (4x6 mL). The organic layer was washed with brine and dried over anhydrous magnesium sulfate. After filtration and removal of solvent, a yellow solid was obtained. Crude product was collected and dissolved in ethyl acetate in an oil bath. The solution was kept in the refrigerator overnight and the solid was filtered off to give 110 mg of light yellow/pale orange solid, resulting a 53% yield. \(^1\)H (400 MHz, CDCl\(_3\)) \(\delta\) ppm 7.22-7.26 (m, 1H), 7.41 (s, 1H), 7.99 (d, \(J=8.1\) Hz, 1H), 8.36 (d, \(J=5.1\) Hz, 1H). LCMS found 196.9, 198.9 [M + H]\(^+\).
3-bromo-1-tosyl-1H-pyrrolo[2,3-b]pyridine (4). DCM (1.9 mL) was added to 3-bromo-1-tosyl-1H-pyrrolo[2,3-b]pyridine (37.7 mg, 0.107 mmol, 70.5 % yield) followed by the addition of TEA (31.8 µl, 0.228 mmol), DMAP (3.72 mg, 0.030 mmol), and TsCl (31.9 mg, 0.167 mmol). The reaction was heated to 80 °C for 3h then cooled to room temperature. The solution turned dark red after heating. The reaction was then stirred at room temperature for 24h. The mixture was washed with 1M HCl, sodium bicarbonate, then brine. The organic layer was concentrated and the product was isolated with chromatography (5-20% ethyl acetate in hexane) to give 37.7 mg white solid in 71% yield. $^1$H (400 MHz, CDCl$_3$) δ ppm 2.39 (s, 3H), 7.26-7.30 (m, 3H), 7.80 (s, 1H), 7.81 (d, $J=8.1$ Hz, 1H), 8.08 (d, $J=7.3$ Hz, 2H), 8.48 (d, $J=4.4$ Hz, 1H). LCMS found 350.9, 352.9 [M + H]$^+$.

N-(2-hydroxy-1-phenylethyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzamide (5). 4-bromo-N-(2-hydroxy-1-phenylethyl)benzamide (100 mg, 0.312 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (79 mg, 0.31 mmol), and potassium acetate (92 mg, 0.94 mmol) were added to a dry vial followed by dry THF (7.6 ml). The mixture was then degassed for 30 min. Pd(dpff)Cl$_2$ DCM adduct (13.7 mg, 0.019 mmol) was then added to the mixture
which was subsequently heated to 80 °C overnight. Solids were removed by filtration using celite, and the filtrate was concentrated. The residue was triturated with hexane and filtered, giving 104 mg brown solid in 91% yield. $^1$H (400 MHz, CDCl$_3$) δ ppm 1.35 (s, 12H), 4.03 (m, 2H), 5.28 (m, 1H), 6.83 (m, 1H), 7.31-7.39 (m, 5H), 7.78-7.80 (m, 2H), 7.86-7.88 (m, 2H). LCMS found 368.1 [M + H]$^+$. 

$\text{N}$(2-hydroxy-1-phenylethyl)-4-(1-tosyl-1H-pyrrolo[2,3-b]pyridin-3-yl)benzamide (NEU-1051).

$\text{N}$(2-hydroxy-1-phenylethyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzamide (59.8 mg, 0.163 mmol), 3-bromo-1-tosyl-1H-pyrrolo[2,3-b]pyridine (57.2 mg, 0.163 mmol), sodium carbonate (37.3 mg, 0.352 mmol), and tetrakis (7.15 mg, 6.19 µmol) in DME (Ratio: 2.69, Volume: 786 µl) and water (Ratio: 1, Volume: 0.3 mL) were degassed for 20 min. The mixture was then heated to 110 °C overnight in a sealed tube. The reaction was extracted with ethyl acetate and water. The organic layer was washed with brine and dried with sodium sulfite. The solvents were evaporated and the residue was separated with chromatography (50-100% EA in hexane). A second column (10-20% EA in DCM) gave 30 mg product in 36% yield. $^1$H (400 MHz, CDCl$_3$) δ ppm 2.39 (s, 3H), 4.05-4.16 (m, 2H), 5.34-5.38 (m, 1H), 7.00 (d, $J$=7.3 Hz, 1H), 7.22-7.36 (m, 4H), 7.40-7.45 (m, 4H), 7.59 (d, $J$=8.1 Hz, 2H), 7.85 (d, $J$=8.1 Hz, 2H), 7.93 (s,
1H), 8.04 (d, J=8.1 Hz, 1H), 8.12 (d, J=8.1 Hz, 2H), 8.46 (d, J=4.4 Hz, 1H). LCMS found 512.1 [M + H]⁺.

N-(2-hydroxy-1-phenylethyl)-4-(1H-pyrrolo[2,3-b]pyridin-3-yl)benzamide (NEU-1052). To N-(2-hydroxy-1-phenylethyl)-4-(1-tosyl-1H-pyrrolo[2,3-b]pyridin-3-yl)benzamide (15.7 mg, 0.031 mmol) in dioxane (0.83 mL) was added NaOH (41.0 µl, 0.082 mmol). The reaction was heated to 110 °C for 2 hours. The reaction turned clear when heated then yellow. The solvents were evaporated, water was added, and crude product was extracted with ethyl acetate. The organic layer was washed with brine and dried over sodium sulfate. The solvent was removed and the product was separated by HP-LC fraction links (5-50% ACN) to give 5 mg white solid in 46% yield. ¹H (500 MHz, DMSO-d6) δ ppm 3.65 (m, 1H), 3.72-3.77 (m, 1H), 4.93 (t, J=5.9 Hz, 1H), 5.08-5.12 (m, 1H), 7.17-7.20 (dd, J=4.9, 7.8 Hz, 1H), 7.22-7.25 (m, 1H), 7.31-7.34 (t, J=7.6 Hz, 2H), 7.4 (d, J=7.3 Hz, 2H), 7.82 (d, J=8.3 Hz, 2H), 7.98 (m, 2H), 8.01 (d, J=2.9 Hz, 1H), 8.29 (m, 1H), 8.34 (d, J=8.1 Hz, 1H), 8.66 (d, J=8.3 Hz, 1H), 12.03 (s, 1H). LCMS found 358.0 [M + H]⁺.
7-hydroxy-1H-pyrrolo[2,3-b]pyridin-7-iium 3-chlorobenzoate (10). 1H-pyrrolo[2,3-b]pyridine (5.00 g, 42.3 mmol) in DME/hexane (Ratio: 1:2, Volume: 77 ml) was stirred in an ice bath followed by the portionwise addition of mCPBA (11.7 g, 67.8 mmol). The reaction stirred at room temperature for 2 hours. The product was filtered and washed with DME/hexane to give a beige solid with 90% yield. $^1$H (400 MHz, DMSO-d$_6$) δ ppm 6.58 (m, 1H), 7.04-7.08 (m, 1H), 7.45 (m, 1H), 7.52-7.57 (m, 1H), 7.63 (d, $J$=8.1 Hz, 1H), 7.70 (d, $J$=8.1 Hz, 1H), 7.89 (m, 2H), 8.11 (d, $J$=5.4 Hz, 1H), 12.45 (s, 1H).

![Chemical Structure](image)

4-chloro-1H-pyrrolo[2,3-b]pyridine (11). To 7-hydroxy-1H-pyrrolo[2,3-b]pyridin-7-iium 3-chlorobenzoate (2.02 g, 6.96 mmol) was added POCl$_3$ (7.78 ml, 84.0 mmol) at room temperature. The mixture was heated to 55 °C then heat was turned off but flask remained in oil bath. The reaction stirred for 1 hour then was heated to 90 °C overnight. Excess phosphoryl chloride was distilled off and the reaction was cooled to room temperature then quenched with sodium bicarbonate and 1M NaOH. The solution was then extracted with DCM and washed with brine. The organic layer was dried over sodium sulfate and concentrated. Product was separated by column chromatography (10-30% ethyl acetate in hexane) to give 800 mg of off-white in 75% yield. $^1$H (400 MHz, DMSO-d$_6$) δ ppm 7.49-7.52 (m, 1H), 7.19 (d, $J$=5.1 Hz, 1H), 7.60 (m, 1H), 8.17 (d, $J$=5.1 Hz, 1H), 12.05 (s, 1H). LCMS found 153.0 [M + H]$^+$. 
4-chloro-1-tosyl-1H-pyrrolo[2,3-b]pyridine (12). To 4-chloro-1H-pyrrolo[2,3-b]pyridine (3.24 g, 21.2 mmol) in DCM (260 ml) was added TEA (6.66 ml, 47.8 mmol), DMAP (0.778 g, 6.37 mmol), then TsCl (6.66 g, 34.9 mmol). The reaction stirred at room temperature overnight. The solution was washed with 1M HCl, sodium bicarbonate, and then brine. The organic layer was dried over sodium sulfate and the solvent evaporated. The residue was separated by column chromatography (60-80% DCM in hexane) to give 6.06 g white solid in 93% yield. $^1$H (400 MHz, DMSO-d$_6$) δ ppm 2.35 (s, 3H), 6.88 (d, $J$=4.4 Hz, 1H), 7.42 (d, $J$=8.1 Hz, 2H), 7.47 (d, $J$=5.1 Hz, 1H), 7.99 (d, $J$=8.8 Hz, 2H), 8.03 (d, $J$=4.4 Hz, 1H), 8.34 (d, $J$=5.1 Hz, 1H).

4-(furan-2-yl)-1-tosyl-1H-pyrrolo[2,3-b]pyridine (13). To 4-chloro-1-tosyl-1H-pyrrolo[2,3-b]pyridine (1.001 g, 3.27 mmol), furan-2-ylboronic acid (0.733 g, 6.55 mmol), potassium fluoride (0.628 g, 10.8 mmol), tris(dibenzylideneacetone)dipalladium(0) (0.045 g, 0.049 mmol), and dioxane (33 mL) was added followed by tri-t-butylphosphine (0.038 ml, 0.147 mmol). The reaction was heated to 100 °C overnight. Water was added after the solution cooled and the organic layer was extracted with ethyl acetate then dried over sodium sulfate. Solvent was removed and the product was collected by column chromatography (5-20% ethyl acetate in hexane) to give 927 mg beige solid in 84% yield. $^1$H (400 MHz, DMSO-d$_6$) δ ppm 2.34 (s, 3H),
6.74 (m, 1H), 7.24 (d, J=2.9 Hz, 1H), 7.42-7.44 (m, 3H), 7.58 (d, J=5.1 Hz, 1H), 7.98-8.01 (m, 4H), 8.36 (d, J=5.1 Hz, 1H). LCMS found 339.1 [M + H]⁺.

4-(5-nitrofuran-2-yl)-1-tosyl-1H-pyrrolo[2,3-b]pyridine (14). Acetic anhydride (207 µl, 2.19 mmol) was cooled to -30 °C in an acetone/dry ice bath. One drop of fuming nitric acid (15.53 µl, 0.348 mmol) was added while maintaining the temperature. 4-(furan-2-yl)-1-tosyl-1H-pyrrolo[2,3-b]pyridine (70.0 mg, 0.207 mmol) in 2 mL acetic anhydride (207 µl, 2.19 mmol) was added dropwise and the reaction stirred for 4 h at -30 °C. The reaction stirred at room temperature overnight. Reaction mixture was poured into ice then saturated sodium bicarbonate was added until solution reached a pH of 6. The mixture was extracted with ethyl acetate and dried over magnesium sulfate. The solvents were removed and the residue was separated by chromatography (10-20% EA in hexanes) to give 7 mg yellow solid in 9% yield. ¹H (400 MHz, DMSO-d₆) δ ppm 2.31 (s, 3H), 7.25 (d, J=3.7 Hz, 1H), 7.39 (d, J=8.8 Hz, 2H), 7.71 (d, J=5.1 Hz, 1H), 7.97 (d, J=8.1 Hz, 2H), 8.05 (m, 2H), 8.41 (d, J=5.1 Hz, 1H), 9.20 (s, 1H). LCMS found 384.1 [M + H]⁺.
5-(1-tosyl-1H-pyrrolo[2,3-b]pyridin-4-yl)furan-2-carbaldehyde (15). To 4-chloro-1-tosyl-1H-pyrrolo[2,3-b]pyridine (190 mg, 0.619 mmol), (5-formylfuran-2-yl)boronic acid (173 mg, 1.24 mmol), potassium fluoride (119 mg, 2.04 mmol), tris(dibenzylideneacetone)dipalladium(0) (28 mg, 0.031 mmol), and degassed dioxane (Volume: 6.2 mL) was added followed by tri-t-butylphosphine (16 µl, 0.062 mmol). The reaction was heated to 100°C overnight. Water was added after the solution cooled and the organic layer was extracted with ethyl acetate then dried over sodium sulfate. The product was collected by column chromatography (5-60% ethyl acetate in hexane) to give 137 mg yellow solid in 60% yield. \(^1\)H (400 MHz, DMSO-d\(_6\)) \(\delta\) ppm 2.34 (s, 3H), 7.30 (d, J=4.4 Hz, 1H), 7.42 (d, J=8.1 Hz, 2H), 7.68 (d, J=4.4 Hz, 1H), 7.73 (d, J=3.7 Hz, 1H), 7.77 (d, J=5.1 Hz, 1H), 8.00 (d, J=8.1 Hz, 2H), 8.12 (d, J=4.4 Hz, 1H), 8.45 (d, J=5.1 Hz, 1H), 9.74 (s, 1H). LCMS found 267.1 [M + H]⁺.

4-(5-bromofuran-2-yl)-1-tosyl-1H-pyrrolo[2,3-b]pyridine (18). Bromine (0.298 mL, 5.79 mmol) was added to 4-(furan-2-yl)-1-tosyl-1H-pyrrolo[2,3-b]pyridine (980 mg, 2.90 mmol) in DCE (0.2 M) and the reaction was refluxed for 45 min. The mixture was cooled and the solid filtered. The crude product was partially dissolved in DCM and washed with 10% thiosulfate until the orange color disappeared. The organic layer was dried over sodium sulfate and concentrated. The solid was separated with chromatography (5-20% ethyl acetate in hexanes), giving 730 mg white solid in 61% yield. \(^1\)H (400 MHz, DMSO-d\(_6\)) \(\delta\) ppm 2.34 (s, 3H), 6.88 (d,
$J=3.7 \text{ Hz, } 1\text{H}), \ 7.17 \ (d, \ J=4.4 \text{ Hz, } 1\text{H}), \ 7.42 \ (d, \ J=8.1 \text{ Hz, } 2\text{H}), \ 7.48 \ (d, \ J=3.7 \text{ Hz, } 1\text{H}), \ 7.56 \ (d, \ J=5.9 \text{ Hz, } 1\text{H}), \ 7.99-8.02 \ (m, \ 3\text{H}), \ 8.37 \ (d, \ J=5.1 \text{ Hz, } 1\text{H}).$ LCMS found 417.0, 419.0 [M + H]$^+$. 

[Diagram]

tert-butyl 5-(1-tosyl-1H-pyrrolo[2,3-b]pyridin-4-yl)furan-2-ylcarbamate (17). To tert-butyl carbamate (30.9 mg, 0.264 mmol), 4-(5-bromofuran-2-yl)-1-tosyl-1H-pyrrolo[2,3-b]pyridine (100 mg, 0.240 mmol), palladium (II) acetate (5.4 mg, 0.024 mmol), xantphos (24 mg, 0.042 mmol), and cesium carbonate (156 mg, 0.479 mmol) was added degased anhydrous 1,4-dioxane (Volume: 2.8 mL). The mixture was refluxed for 5 hrs at 120$^\circ$C. Water was added and the product was extracted with ethyl acetate. The organic layer was washed with brine and dried over sodium sulfate. After concentration, the residue was separated using chromatography (5-15% EA in hexanes) to give 20 mg amber oil in 18% yield. $^1\text{H} \ (400 \text{ MHz}, \ \text{DMSO-d}_6) \ \delta \ \text{ppm } 1.46 \ (s, \ 9\text{H}), \ 2.31 \ (s, \ 3\text{H}), \ 6.10 \ (m, \ 1\text{H}), \ 7.32 \ (d, \ J=3.7 \text{ Hz, } 1\text{H}), \ 7.37-7.41 \ (m, \ 4\text{H}), \ 7.91 \ (d, \ 1\text{H}), \ 7.95 \ (d, \ J=8.8 \text{ Hz, } 2\text{H}), \ 8.22 \ (d, \ J=5.1 \text{ Hz, } 1\text{H}).$ LCMS found 454.2 [M + H]$^+$. 

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tert-butyl (1-oxo-3-phenyl-1-((5-(1-tosyl-1H-pyrrolo[2,3-b]pyridin-4-yl)furan-2-yl)amino)propan-2-yl)carbamate (NEU-2126). To CuI (2.74 mg, 0.014 mmol), potassium carbonate (0.085 g, 0.618 mmol), and tert-butyl (1-amino-1-oxo-3-phenylpropan-2-yl)carbamate (0.057 g, 0.216 mmol) was added degassed dioxane (0.3 mL). N,N'-dimethylethylenediamine (1.548 µL, 0.014 mmol) was added followed by the dropwise addition of 4-(5-bromofuran-2-yl)1-tosyl-1H-pyrrolo[2,3-b]pyridine (60.0 mg, 0.144 mmol) in 0.5 mL dioxane. The reaction was placed in a preheated oil bath at 110 °C overnight. The reaction mixture was filtered through a short silica plug with DCM to remove the starting material then the silica was flushed with ethyl acetate to elude the product. The solvents were removed and the residue separated with chromatography (15-40% ethyl acetate in hexanes). A light yellow solid was obtained. The crude product was purified using the prep HPLC to give 27 mg of white solid in 32% yield. $^1$H (500 MHz, DMSO-d$_6$) δ ppm 2.34 (s, 3H), 2.82-2.87 (m, 1H), 2.99-3.03 (m, 1H), 4.34 (m, 1H), 6.41 (d, $J$=3.4 Hz, 1H), 7.20 (m, 1H), 7.27-7.30 (m, 3H), 7.32-7.35 (m, 3H), 7.40-7.43 (m, 3H), 7.47 (d, $J$=5.4 Hz, 1H), 7.98-8.00 (m, 3H), 8.29 (d, $J$=5.4 Hz, 1H), 11.49 (s, 1H). LCMS found 601.0 [M + H]$^+$. 
2-amino-3-phenyl-N-(5-(1-tosyl-1H-pyrrolo[2,3-b]pyridin-4-yl)furan-2-yl)propanamide (NEU-2127). To tert-butyl (1-oxo-3-phenyl-1-((5-(1-tosyl-1H-pyrrolo[2,3-b]pyridin-4-yl)furan-2-yl)amino)propan-2-yl)carbamate (20.0 mg, 0.033 mmol) in MeOH (1 mL) was added 1M HCl in dioxane (0.55 mL). The reaction stirred at room temperature for 4 hours, turning yellow upon addition of HCl then amber after stirring. The solvents were removed and the residue was dissolved in EA and washed with a sodium bicarbonate solution then brine. The organic layer was dried over sodium sulfate and concentrated. The residue was separated by chromatography (30-80% EA in hexane). The crude product was further purified by a second column (1-5% MeOH, DCM). Due to a small amount of impurity present, the product was purified with HPCL fraction links (5-95% gradient, basic mode) to give 9.3 mg light brown solid in 56% yield. $^1$H (500 MHz, DMSO-d$_6$) δ ppm 2.34 (s, 3H), 2.72-2.77 (m, 1H), 2.99-3.02 (m, 1H), 3.62-3.64 (m, 1H), 6.42 (d, J=3.9 Hz, 1H), 7.18 (m, 1H), 7.24-7.30 (m, 5H), 7.36 (d, J=3.9 Hz, 1H), 7.40 (d, J=3.9 Hz, 1H), 7.41 (d, J=8.3 Hz, 2H), 7.46 (d, J=4.9 Hz, 1H), 7.97-8.00 (m, 3H), 8.28 (d, J=4.9 Hz, 1H). LCMS found 501.1 [M + H]$^+$. 
N-(5-(1H-pyrrolo[2,3-b]pyridin-4-yl)furan-2-yl)-2-amino-3-phenylpropanamide (NEU-2139). To 2-amino-3-phenyl-N-(5-(1-tosyl-1H-pyrrolo[2,3-b]pyridin-4-yl)furan-2-yl)propanamide (9.0 mg, 0.018 mmol) in MeOH (0.28 mL) was added 6N NaOH (0.28 mL). The reaction was heated to 55 °C for 30 min. The reaction was neutralized with 1M HCl and extracted with DCM and ethyl acetate. The organic layer was dried over sodium sulfate and the solvents were removed. The residue was purified by prep TLC (10% MeOH in EA) then with HPLC fraction links (basic mode). $^1$H (500 MHz, CD$_3$OD) δ ppm 2.97 (m, 1H), 3.14-3.18 (m, 1H), 3.87 (m, 1H), 6.52 (d, $J$=3.4 Hz, 1H), 6.92 (d, $J$=3.4 Hz, 1H), 7.17 (d, $J$=3.4 Hz, 1H), 7.23-7.36 (m, 6H), 7.43 (d, $J$=3.4 Hz, 1H), 8.31 (d, $J$=4.9 Hz, 1H). LCMS found 347.1 [M + H]$^+$. 

(tert-butoxycarbonyl)phenylalanine (25). Sodium carbonate (1.30 g, 12.1 mmol) was added to a mixture of L-phenylalaline (1.00 g, 6.05 mmol) in water:THF (10:1, 8.0 mL) in an ice bath, followed by the addition of Boc$_2$O (1.45 g, 6.66 mmol). The reaction stirred at room temperature for 1 day. With 3M HCl, the mixture was acidified to a pH of 2 then extracted with ethyl
acetate, washed with brine, and dried over sodium sulfate. The solvents were removed with reduced pressure to give 1.60 g yellow oil, 100% yield. $^1$H (400 MHz, DMSO-d$_6$) $\delta$ ppm 1.31 (s, 9H), 2.78-2.84 (dd, $J$=10.3, 13.9 Hz, 1H), 2.98-3.03 (dd, $J$=4.8, 13.6 Hz, 1H), 4.05-4.11 (m, 1H), 7.10 (d, $J$=8.1 Hz, 1H), 7.19-7.29 (m, 5H).

**tert-butyl (1-amino-1-oxo-3-phenylpropan-2-yl)carbamate (26).** To (tert-butoxycarbonyl)phenylalanine (415 mg, 1.56 mmol) in THF (1 mL) was added TEA (0.240 mL, 1.72 mmol) and the reaction stirred at -15 °C. Ethyl chloroformate (0.165 mL, 1.72 mmol) in THF (1.6 mL) was added dropwise and the reaction stirred for 25 min followed by the addition of ammonium hydroxide. The reaction stirred for 3h between 0-5 °C. After completion, the pH was adjusted to 2-3 and the mixture was extracted with ethyl acetate. The organic layer was washed with saturated sodium bicarbonate three times, brine, and then dried over sodium sulfate. The solvents were removed to give 324 mg white solid in 78% yield. $^1$H (400 MHz, CDCl$_3$) $\delta$ ppm 1.42 (s, 9H), 3.09 (m, 1H), 4.37 (m, 2H), 5.04 (m, 1H), 5.27 (bs, 1H), 5.72 (bs, 1H), 7.24-7.35 (m, 5H). LCMS found 287.1 [M + H]$^+$.  

**tert-butyl (1-amino-4-methyl-1-oxopentan-2-yl)carbamate (40).** To boc-L-leucine (200 mg, 0.865 mmol) in THF (0.5 mL) was added TEA (0.133 mL, 0.951 mmol) and the reaction stirred...
at -15 °C. Ethyl chloroformate (0.087 mL, 0.908 mmol) in THF (0.8 mL) was added dropwise and the reaction stirred for 25 min followed by the addition of ammonium hydroxide (0.277 mL, 3.72 mmol). The reaction stirred for 3 h between 0-5 °C. The pH was adjusted to 2-3 using 1M KHSO$_4$ and the mixture was extracted with ethyl acetate. The organic layer was washed with saturated sodium bicarbonate three times, once with brine, then dried over sodium sulfate. The solvents were removed to give 141 mg white solid in 71% yield. $^1$H (500 MHz, CDCl$_3$) δ ppm 0.96 (t, $J$=5.5 Hz, 6H), 1.46 (s, 9H), 1.48-1.52 (m, 1H), 1.67-1.74 (m, 2H), 4.11-4.15 (m, 1H), 4.85 (bs, 1H), 5.36 (bs, 1H), 6.09 (bs, 1H). LCMS found 253.0 [M + H]$^+$. 

1-(tert-butoxycarbonyl)piperidine-3-carboxylic acid (41). In an ice bath, di-tert-butyl dicarbonate (1.00 ml, 4.34 mmol) in THF (Volume: 30 ml) was slowly added dropwise to piperidine-3-carboxylic acid (0.510 g, 3.95 mmol) and sodium carbonate (1.57 g, 14.8 mmol) in THF and water (Ratio: 1:1, Volume: 200 ml). The reaction stirred for 2 h at 0 °C then overnight at room temperature. The THF was evaporated and the aqueous solution was acidified with 3M HCl (to pH of about 3-4). White precipitate was visible. The product was extracted with ethyl acetate and the organic layer was dried over sodium sulfate and concentrated. The product was then separated with chromatography (1-5% MeOH in DCM) to give 778 mg white solid in 86% yield. $^1$H (400 MHz, DMSO-d$_6$) δ ppm 1.34 (m, 1H), 1.39 (s, 9H), 1.50 (m, 1H), 1.60 (m, 1H), 1.90 (m, 1H), 2.29 (m, 1H), 2.83 (m, 2H), 3.68 (m, 1H), 3.89 (m, 1H), 13.36 (s, 1H). LCMS found 252.1 [M + H]$^+$. 

![Chemical structure of 41]
ethyl piperidine-3-carboxylate hydrochloride (42). To piperidine-3-carboxylic acid (2.00 g, 15.5 mmol) in anhydrous ethanol (Volume: 7.7 ml) was added thionyl chloride (4.18 ml, 57.3 mmol) in an ice bath then refluxed for 5h. Solvents were removed to give 2.92 g brown solid in 97% yield. $^1$H (400 MHz, D$_2$O) δ ppm 1.19 (t, $J$=7.0 Hz, 3H), 1.68-1.76 (m, 2H), 1.80-1.85 (m, 1H), 2.02-2.06 (m, 1H), 2.82-2.88 (m, 1H), 2.97-3.02 (m, 1H), 3.16-3.22 (m, 2H), 3.39-3.41 (m, 1H), 4.09-4.17 (m, 2H).

1-tert-butyl 3-ethyl piperidine-1,3-dicarboxylate (43). To ethyl piperidine-3-carboxylate (0.79 g, 5.00 mmol) in DCM (Volume: 35 ml) was added TEA (1.46 ml, 10.5 mmol) and BOC$_2$O (1.16 ml, 5.00 mmol). The reaction stirred at room temperature overnight. The solution was diluted with 100 mL ethyl acetate and washed twice with 5% HCl, sodium bicarbonate, and brine. The organic layer was dried with sodium sulfate and the solvents evaporated. The residue was separated by column chromatography (5-15% EA in hexane) to give 1.13 g pale yellow liquid in 88% yield. $^1$H (400 MHz, CDCl$_3$) δ ppm 1.26 (t, $J$=7.3 Hz, 3H), 1.46 (s, 9H), 1.56-1.62 (m, 2H), 1.67-1.73 (m, 2H), 2.02-2.08 (m, 1H), 2.40-2.46 (m, 1H), 2.76-2.83 (m, 1H), 2.91-3.00 (m, 1H), 3.90-3.96 (m, 1H), 4.11 (q, $J$=7.3 Hz, 2H). LCMS found 280.1 [M + H]$^+$. 

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1-tert-butyl 3-ethyl 3-benzylpiperidine-1,3-dicarboxylate (44). To LDA (1.6 ml, 3.20 mmol) was added 1-tert-butyl 3-ethyl piperidine-1,3-dicarboxylate (374 mg, 1.46 mmol) dissolved in about THF (1.8 mL) dropwise and the solution stirred for 30 minutes under a nitrogen atmosphere. (bromomethyl)benzene (0.190 ml, 1.60 mmol) in about 1 mL THF was added dropwise and stirred in the dry ice bath, which was gradually warmed to room temperature and stirred over the weekend. The solution was diluted with DCM and washed with water, twice with sodium bicarbonate, again with water, and finally brine. The organic layer was dried over magnesium sulfate and concentrated. The product was separated by chromatography (1-15% EA in hexanes) to give 300 mg clear liquid in 59% yield. $^1$H (400 MHz, CDCl$_3$) δ ppm 1.17 (t, 3H), 1.46 (s, 9H), 1.50-1.69 (m, 3H), 2.00-2.06 (m, 1H), 2.74 (d, $J$=13.9 Hz, 1H), 2.92 (d, $J$=13.2 Hz, 1H), 3.13 (m, 2H), 3.56-3.62 (m, 1H), 4.05 (m, 3H), 7.09-7.11 (m, 2H), 7.21-7.28 (m, 3H). LCMS found 348.2 [M + H]$^+$.  

3-benzyl-1-(tert-butoxycarbonyl)piperidine-3-carboxylic acid (45). To 1-tert-butyl 3-ethyl 3-benzylpiperidine-1,3-dicarboxylate (67 mg, 0.193 mmol) in ethanol (Volume: 0.35 mL) was
added aqueous NaOH (0.329 mL, 1.317 mmol). The reaction was refluxed for 1 day. The solvents were evaporated and water was added. In an ice bath, the solution was acidified to pH of 3 using 1M HCl. The product was extracted with ethyl acetate, washed with water, brine, and dried over sodium sulfate. The solvents were removed to give 52 mg clear oil in 88% yield. \(^1\)H (400 MHz, CD\(_3\)OD) \(\delta\) ppm 1.43 (s, 9H), 1.48-1.66 (m, 4H), 2.00 (m, 1H), 2.75 (d, \(J=13.2\) Hz, 1H), 2.90 (d, \(J=13.2\) Hz, 1H), 3.06 (m, 1H), 3.66 (d, \(J=12.5\) Hz, 1H), 4.10 (m, 1H), 7.92-8.09 (m, 5H), 13.27 (s, 1H). LCMS found 320.2 [M + H]+.

2-(hydroxymethyl)isoindoline-1,3-dione (46). Isoindoline-1,3-dione (1.54 g, 10.4 mmol) and formaldehyde (2.91 ml, 39.1 mmol) in water (Volume: 3.07 ml) were stirred at 100 °C for 4 hours. The solid was filtered and washed with water and hexane to give 1.77 g white solid in 96% yield. \(^1\)H (400 MHz, CDCl\(_3\)) \(\delta\) ppm 3.52 (s, 1H), 5.27 (s, 2H), 7.77-7.79 (m, 2H), 7.91-9.93 (m, 2H).

2-(bromomethyl)isoindoline-1,3-dione (47). 2-(hydroxymethyl)isoindoline-1,3-dione (1.77 g, 9.97 mmol) and HBr (10.3 ml, 62.4 mmol) were stirred in an ice bath. Sulfuric acid (2.66 ml, 31.9 mmol) was then added dropwise. The reaction was then heated to 65 °C for 5 hours. After heating, the reaction was placed in refrigerator overnight. The solid was filtered and washed with cold water, cold 10% ammonium hydroxide until the filtrate was basic, and again with
water to give 1.72 g white solid in 72% yield. $^1$H (400 MHz, CDCl$_3$) δ ppm 5.50 (s, 2H), 7.78-7.72 (m, 2H), 7.92-7.96 (m, 2H).

**diethyl 2-benzyl-2-((1,3-dioxoisooindolin-2-yl)methyl)malonate (48).** To NaH (0.258 g, 6.46 mmol) in THF (Volume: 24 ml) was added diethyl 2-benzylmalonate (1.41 ml, 5.97 mmol). The solution was stirred for 30 min at room temperature followed by the addition of 2-(bromomethyl)isoindoline-1,3-dione (1.72 g, 7.17 mmol). The reaction was stirred overnight. Water was added and the solution was diluted with ether. After separation, the organic layer was concentrated. The residue was recrystallized in ethanol to give 2.01 g white solid in 82% yield. $^1$H (400 MHz, CDCl$_3$) δ ppm 1.17 (t, $J=7.3$ Hz, 6H), 3.29 (s, 2H), 4.06-4.16 (m, 4H), 4.36 (s, 2H), 7.22-7.29 (m, 3H), 7.39 (d, $J=6.6$ Hz, 2H), 7.73-7.75 (m, 2H), 7.86-7.88 (m, 2H). LCMS found 410.2 [M + H]$^+$.  

**2-benzyl-3-ethoxy-3-oxopropanoic acid (49).** Diethyl benzyl malonate (2 mL, 8.50 mmol) was added to KOH (0.477 g, 8.50 mmol) in ethanol (13 mL). The reaction stirred at room temperature overnight. The solvents were removed and the residue was dissolved in 5% sodium bicarbonate. The starting material was extracted with ethyl acetate. The aqueous layer was
acidified with 1M HCl to pH of 1 and the product was extracted with ethyl acetate. The organic layer was washed with 1M HCl and dried over magnesium sulfate. The solvents were removed to give 1.52 g pale yellow oil in 80% yield. $^1$H (400 MHz, DMSO-d$_6$) δ ppm 1.08 (t, $J$=7.0 Hz, 3H), 3.00-3.10 (m, 2H), 3.69 (t, $J$=7.7 Hz, 1H), 4.03 (q, $J$=7.3 Hz, 2H), 7.18-7.29 (m, 5H).

**ethyl 2-benzylacrylate (50).** 2-benzyl-3-ethoxy-3-oxopropanoic acid (1.06 g, 4.75 mmol) was cooled in an ice bath. Diethyl amine (0.506 mL, 4.89 mmol) and aqueous formaldehyde (0.495 mL, 6.65 mmol) was added dropwise. After stirring for 6 hours at room temperature, the reaction was diluted with water and extracted with ether. The organic layer was washed with 2M HCl, saturated sodium bicarbonate, then brine. The organic layer was dried over magnesium sulfate and the solvents were removed to give 591 mg light yellow oil in 65% yield. $^1$H (400 MHz, CD$_3$OD) δ ppm 1.23 (t, $J$=7.3 Hz, 3H), 3.62 (s, 2H), 4.14 (q, $J$=6.8 Hz, 2H), 5.52 (d, $J$=1.5 Hz, 1H), 6.19 (s, 1H), 7.16-7.29 (m, 5H).

**ethyl 1,3-dibenzylpyrrolidine-3-carboxylate (51).** To ethyl 2-benzylacrylate (524 mg, 2.75 mmol) and N-(methoxymethyl)-N-(trimethylsilylmethyl)benzylamine (0.916 mL, 3.58 mmol) in DCM in an ice bath was added trifluoroacetic acid (0.015 mL, 0.193 mmol). The reaction stirred
at room temperature for 3h then was heated to 50°C. The mixture was diluted with DCM and washed with sodium bicarbonate followed by brine. The organic layer was dried over sodium sulfate and the solvents were removed. The residue was separated by chromatography (2-15% ethyl acetate in hexane) to give 301 mg light yellow oil in 34% yield. $^1$H (400 MHz, CD$_3$OD) $\delta$ ppm 1.19 (t, $J$=7.3 Hz, 3H), 1.87-1.94 (dt, $J$=6.9 (x2), 13.4 Hz, 1H), 2.27-2.34 (dt, $J$=6.9 (x2), 13.4 Hz, 1H), 2.56 (d, $J$=9.5 Hz, 1H), 2.61-2.70 (m, 2H), 2.95-3.08 (m, 4H), 3.61 (s, 2H), 4.08 (q, $J$=7.3 Hz, 2H), 4.90 (s, 1H), 7.06 (m, 2H), 7.15-7.35 (m, 8H).

**Chapter 5 References**

### 5.1 References.


Appendix

Representative NMR spectra